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Lipshutz et al.

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[45] Date of Patent: *May 23, 2000

[54] **COMPUTER-AIDED PROBABILITY BASE CALLING FOR ARRAYS OF NUCLEIC ACID PROBES ON CHIPS**

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[73] Assignee: **Affymetrix, Inc.**, Santa Clara, Calif.

[*] Notice: This patent is subject to a terminal disclaimer.

[21] Appl. No.: **08/948,896**

[22] Filed: **Oct. 10, 1997**

Related U.S. Application Data

[63] Continuation of application No. 08/528,656, Sep. 14, 1995, Pat. No. 5,733,729.

[51] **Int. Cl.**[7] **C12Q 1/68**; C07H 21/04; G06K 9/18

[52] **U.S. Cl.** **435/6**; 436/497; 536/23.1; 536/24.32; 382/129

[58] **Field of Search** 435/6, 973; 536/23.1, 536/24.3, 24.31, 24.32; 935/78; 436/497; 382/129

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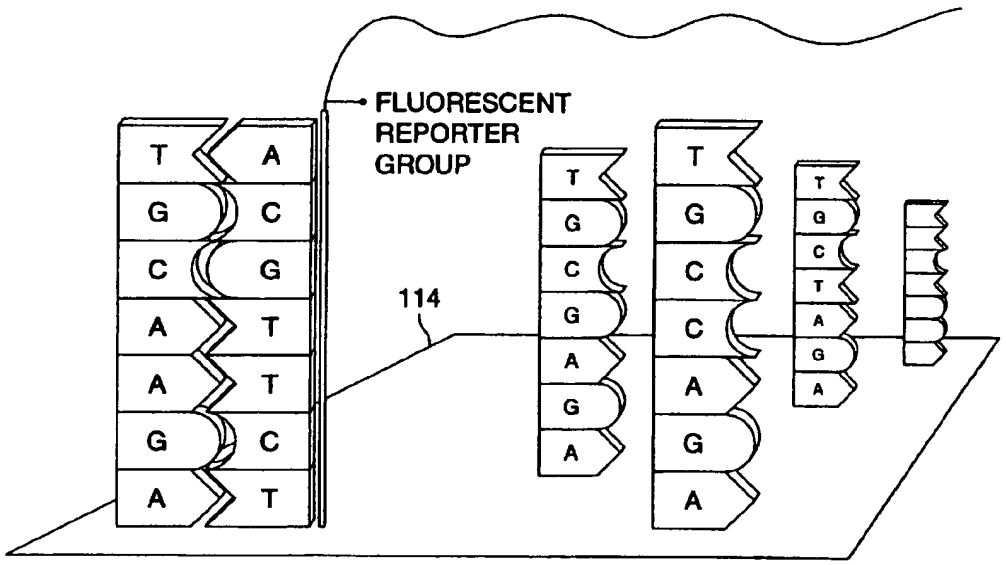
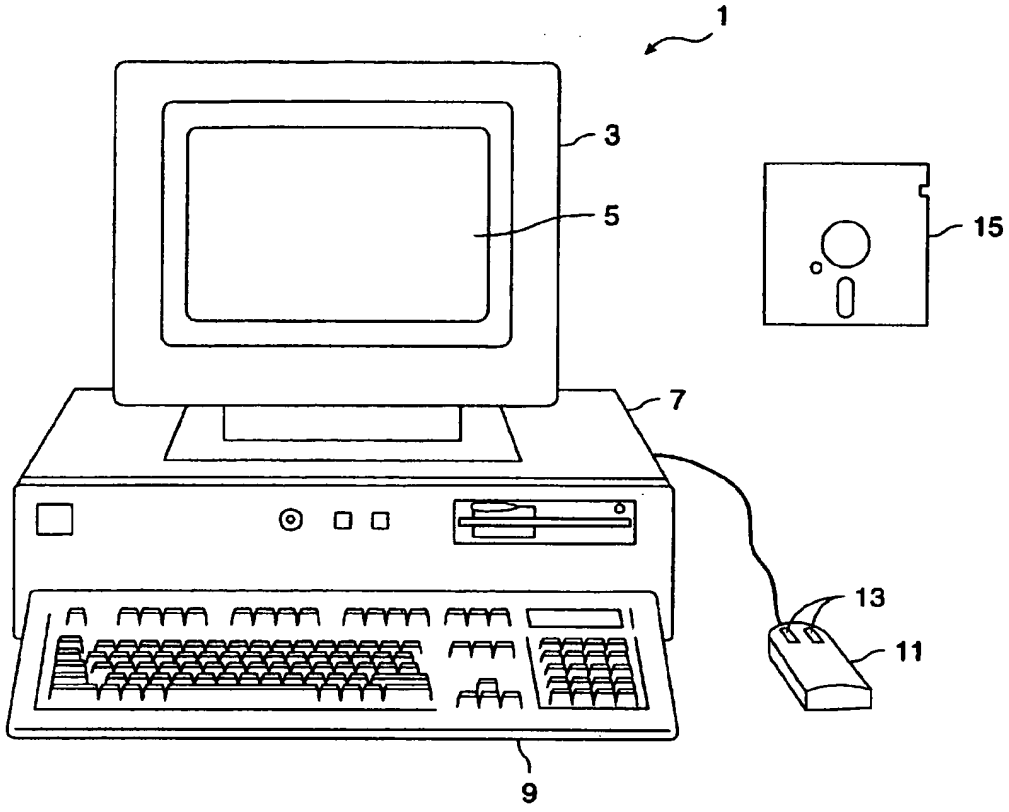
Assistant Examiner—Betty J Forman
Attorney, Agent, or Firm—Ritter, Van Pelt & Yi

[57] **ABSTRACT**

A computer system for analyzing nucleic acid sequences is provided. The computer system is used to calculate probabilities for determining unknown bases by analyzing the fluorescence intensities of hybridized nucleic acid probes on biological chips. Additionally, information from multiple experiments is utilized to improve the accuracy of calling unknown bases.

38 Claims, 8 Drawing Sheets




1
3
5
15
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13
11
9

FIG. 1

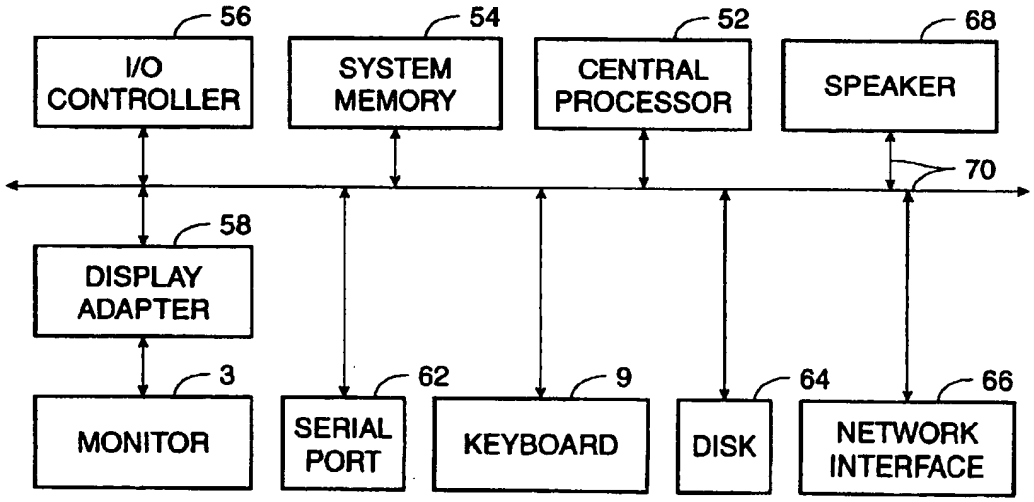
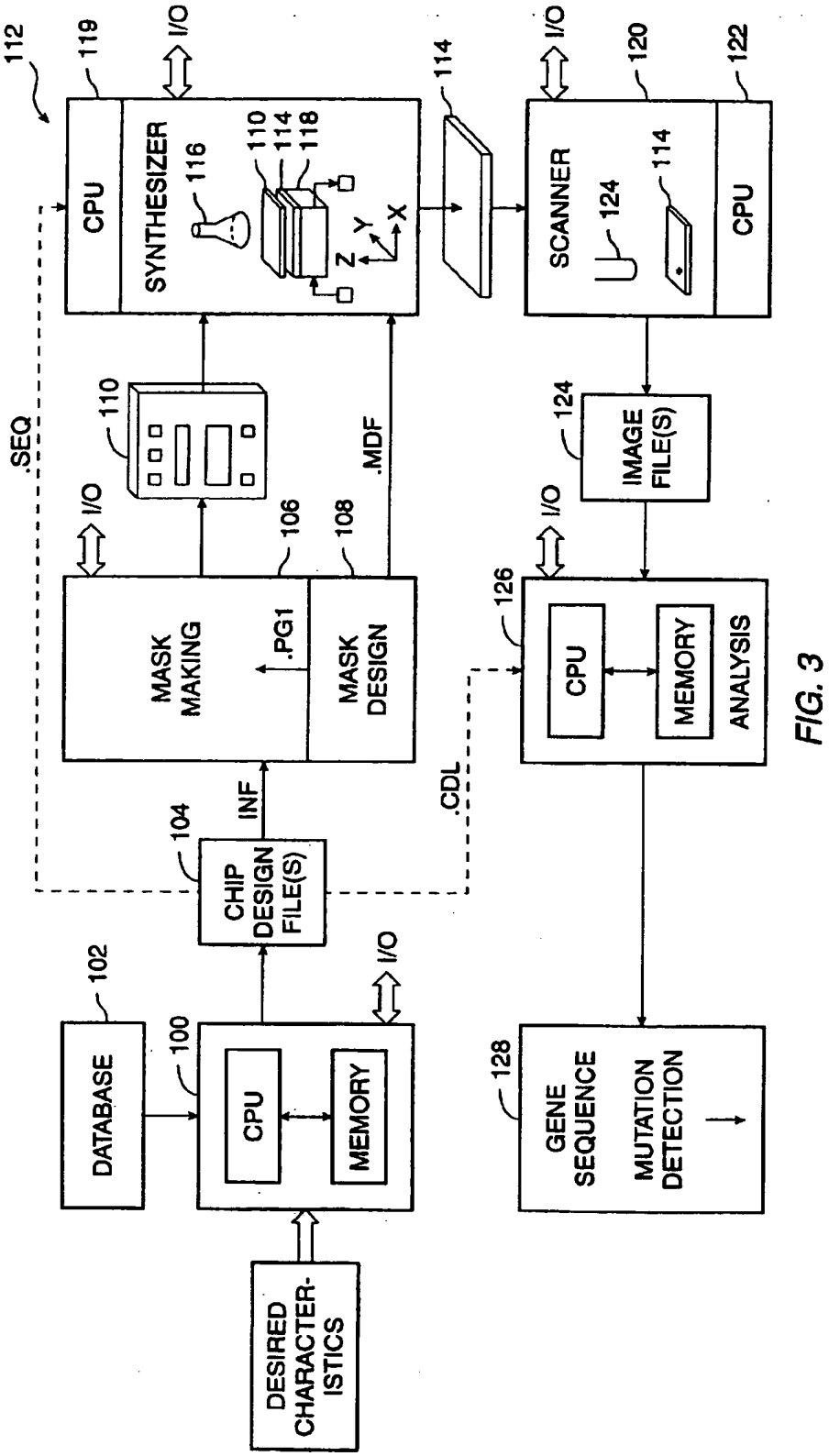

56
I/O CONTROLLER
54
SYSTEM MEMORY
52
CENTRAL PROCESSOR
68
SPEAKER
70
58
DISPLAY ADAPTER
3
MONITOR
62
SERIAL PORT
9
KEYBOARD
64
DISK
66
NETWORK INTERFACE

FIG. 2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114
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.SEQ
110
.MDF
124
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I/O
106
108
I/O
126
MASK MAKING
.PG1
MASK DESIGN
CPU
MEMORY
ANALYSIS
.CDL
INF
104
CHIP DESIGN FILE(S)
102
I/O
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100
CPU
MEMORY
128
GENE SEQUENCE
MUTATION DETECTION
DESIRED CHARACTER-ISTICS

FIG. 3

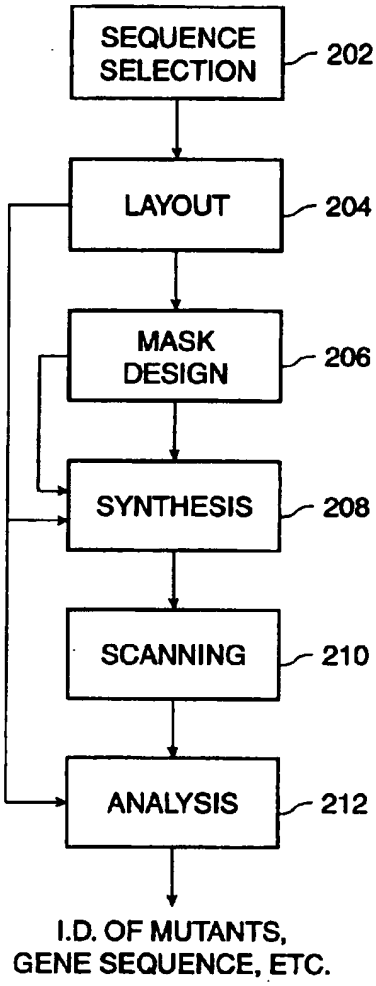
SEQUENCE SELECTION
202
LAYOUT
204
MASK DESIGN
206
SYNTHESIS
208
SCANNING
210
ANALYSIS
212
I.D. OF MUTANTS, GENE SEQUENCE, ETC.

FIG. 4

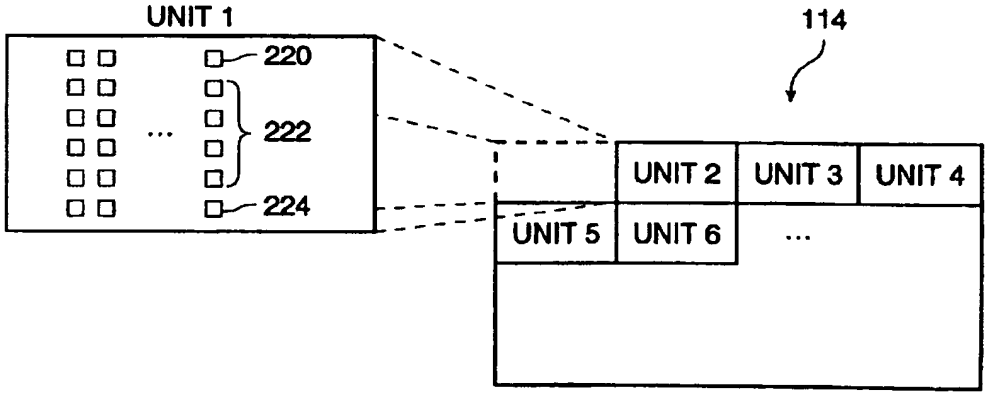
UNIT 1
220
222
224
114
UNIT 2
UNIT 3
UNIT 4
UNIT 5
UNIT 6
...

FIG. 5

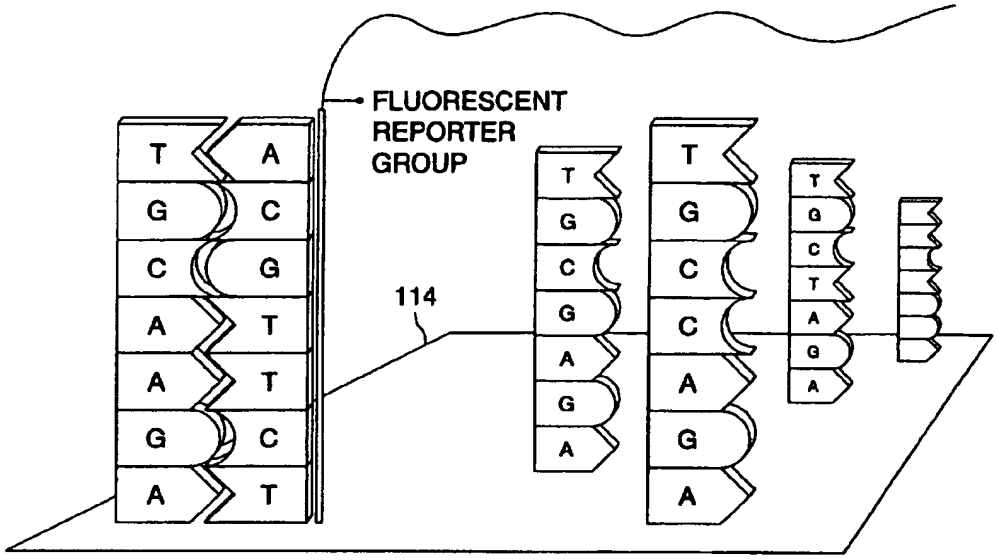

FIG. 6

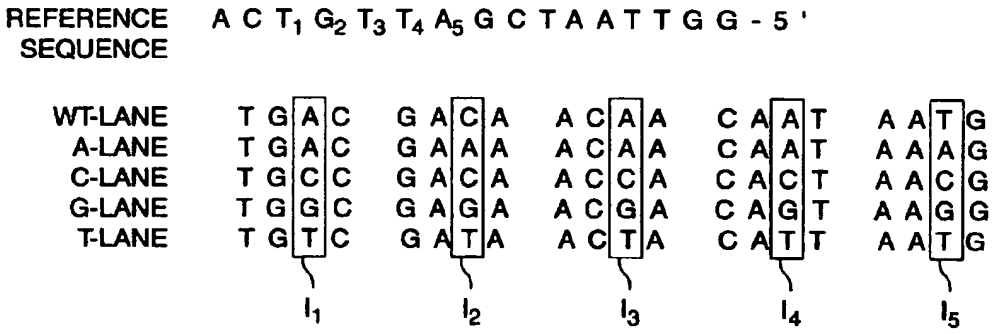

FIG. 7

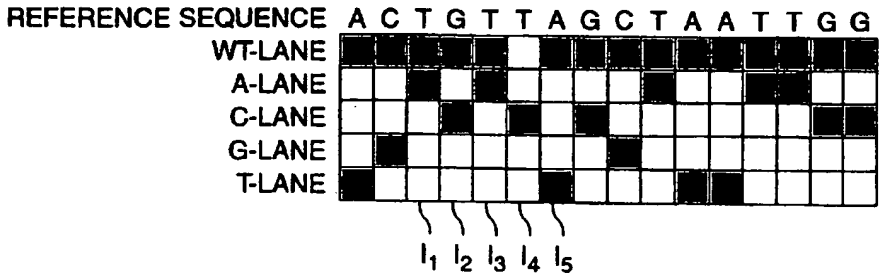

FIG. 8

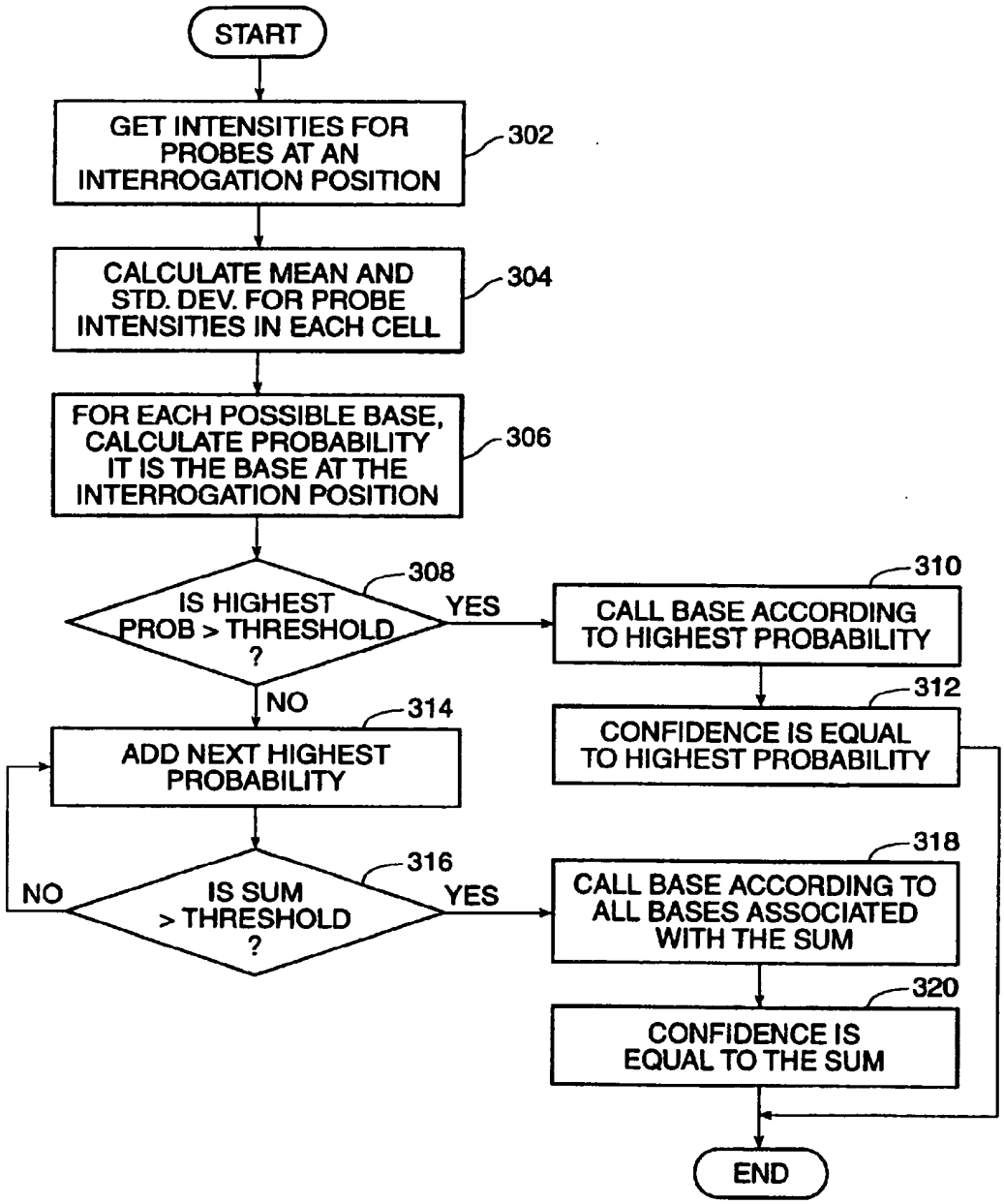
START
GET INTENSITIES FOR PROBES AT AN INTERROGATION POSITION
302
CALCULATE MEAN AND STD. DEV. FOR PROBE INTENSITIES IN EACH CELL
304
FOR EACH POSSIBLE BASE, CALCULATE PROBABILITY IT IS THE BASE AT THE INTERROGATION POSITION
306
IS HIGHEST PROB > THRESHOLD ?
308
YES
NO
CALL BASE ACCORDING TO HIGHEST PROBABILITY
310
CONFIDENCE IS EQUAL TO HIGHEST PROBABILITY
312
ADD NEXT HIGHEST PROBABILITY
314
IS SUM > THRESHOLD ?
316
NO
YES
CALL BASE ACCORDING TO ALL BASES ASSOCIATED WITH THE SUM
318
CONFIDENCE IS EQUAL TO THE SUM
320
END

FIG. 9

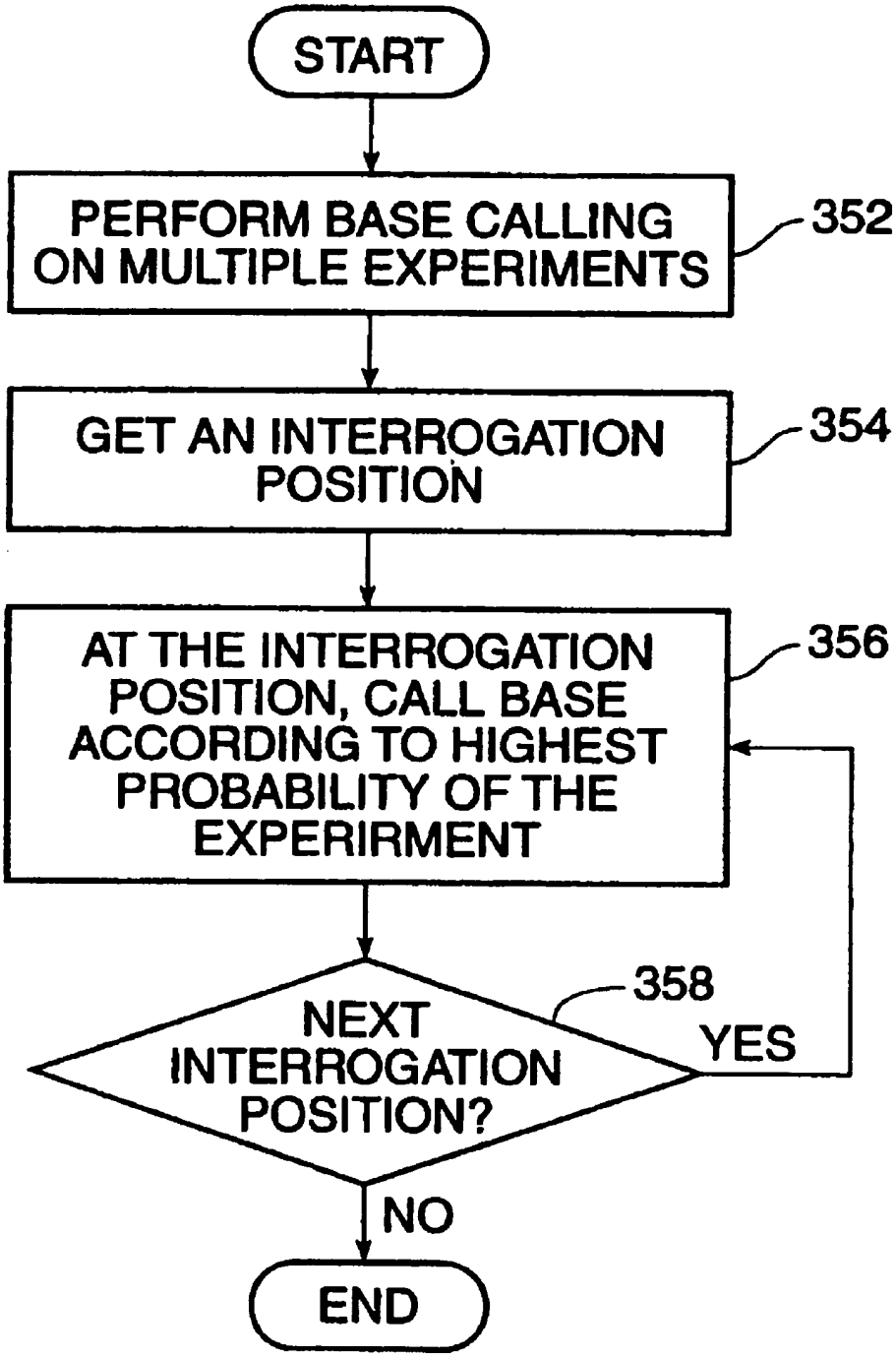
START
PERFORM BASE CALLING ON MULTIPLE EXPERIMENTS
352
GET AN INTERROGATION POSITION
354
AT THE INTERROGATION POSITION, CALL BASE ACCORDING TO HIGHEST PROBABILITY OF THE EXPERIRMENT
356
NEXT INTERROGATION POSITION?
358
YES
NO
END

FIG. 10

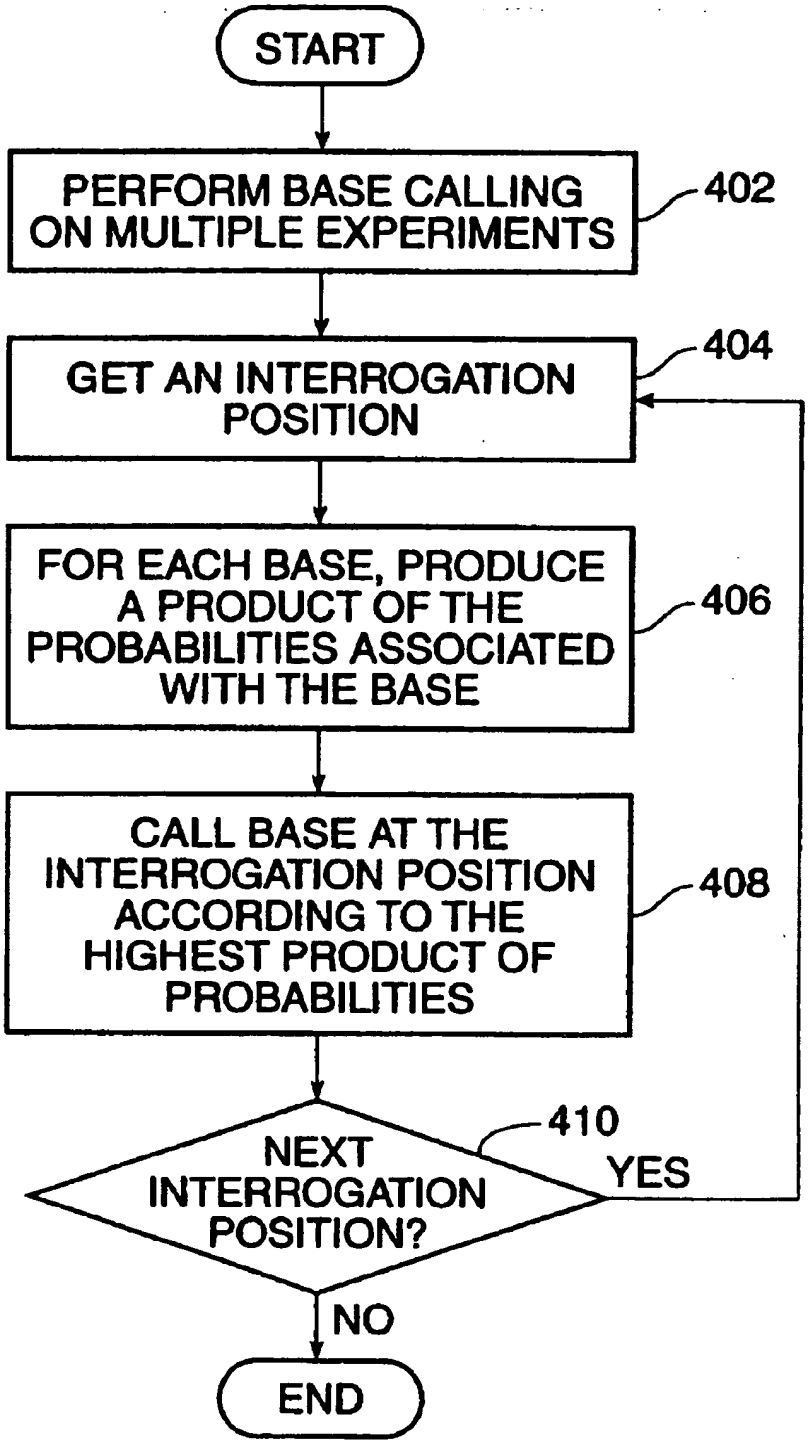
START
PERFORM BASE CALLING ON MULTIPLE EXPERIMENTS
402
GET AN INTERROGATION POSITION
404
FOR EACH BASE, PRODUCE A PRODUCT OF THE PROBABILITIES ASSOCIATED WITH THE BASE
406
CALL BASE AT THE INTERROGATION POSITION ACCORDING TO THE HIGHEST PRODUCT OF PROBABILITIES
408
NEXT INTERROGATION POSITION?
410
YES
NO
END

FIG. 11

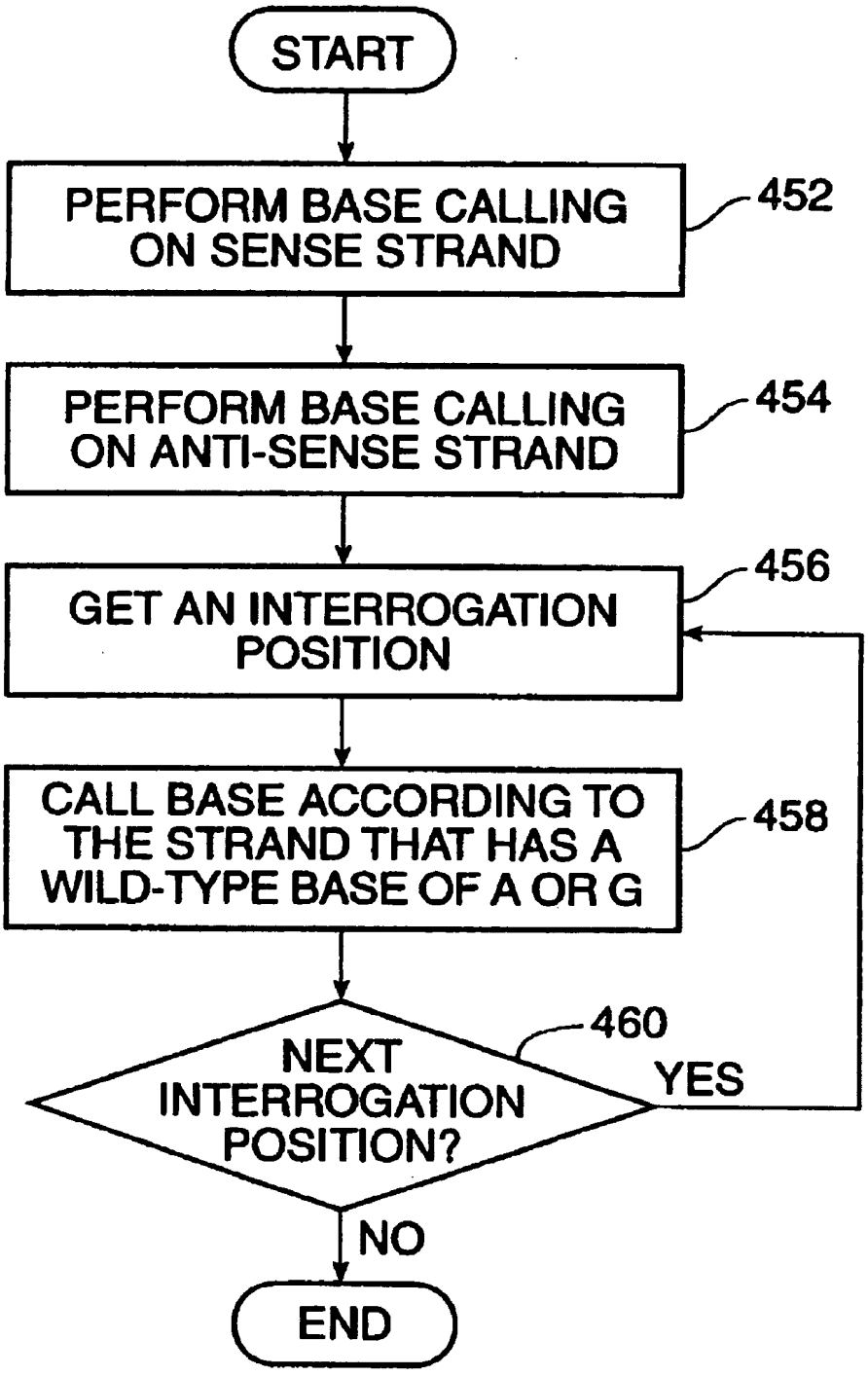
START
PERFORM BASE CALLING ON SENSE STRAND
452
PERFORM BASE CALLING ON ANTI-SENSE STRAND
454
GET AN INTERROGATION POSITION
456
CALL BASE ACCORDING TO THE STRAND THAT HAS A WILD-TYPE BASE OF A OR G
458
NEXT INTERROGATION POSITION?
460
YES
NO
END

FIG. 12

COMPUTER-AIDED PROBABILITY BASE CALLING FOR ARRAYS OF NUCLEIC ACID PROBES ON CHIPS

This is a continuation application of prior application Ser. No. 08/528,656 filed on Sep. 14, 1995, now U.S. Pat. No. 5,733,729, the disclosure of which is incorporated herein by reference.

GOVERNMENT RIGHTS NOTICE

Portions of the material in this specification arose under the cooperative agreement 70NANB5H1031 between Affymetrix, Inc. and the Department of Commerce through the National Institute of Standards and Technology.

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SOFTWARE APPENDIX

A Software Appendix comprising twenty one (21) sheets is included herewith.

BACKGROUND OF THE INVENTION

The present invention relates to the field of computer systems. More specifically, the present invention relates to computer systems for evaluating and comparing biological sequences.

Devices and computer systems for forming and using arrays of materials on a substrate are known. For example, PCT application WO92/10588, incorporated herein by reference for all purposes, describes techniques for sequencing or sequence checking nucleic acids and other materials. Arrays for performing these operations may be formed in arrays according to the methods of, for example, the pioneering techniques disclosed in U.S. Pat. No. 5,143,854 and U.S. patent application Ser. No. 08/249,188, now U.S. Pat. No. 5,571,639, both incorporated herein by reference for all purposes.

According to one aspect of the techniques described therein, an array of nucleic acid probes is fabricated at known locations on a chip or substrate. A fluorescently labeled nucleic acid is then brought into contact with the chip and a scanner generates an image file (also called a cell file) indicating the locations where the labeled nucleic acids bound to the chip. Based upon the image file and identities of the probes at specific locations, it becomes possible to extract information such as the monomer sequence of DNA or RNA. Such systems have been used to form, for example, arrays of DNA that may be used to study and detect mutations relevant to cystic fibrosis, the P53 gene (relevant to certain cancers), HIV, and other genetic characteristics.

Innovative computer-aided techniques for base calling are disclosed in U.S. patent application Ser. No. 08/327,525, which is incorporated by reference for all purposes. However, improved computer systems and methods are still needed to evaluate, analyze, and process the vast amount of information now used and made available by these pioneering technologies.

SUMMARY OF THE INVENTION

An improved computer-aided system for calling unknown bases in sample nucleic acid sequences from multiple nucleic acid probe intensities is disclosed. The present invention is able to call bases with extremely high accuracy (up to 98.5%). At the same time, confidence information may be provided that indicates the likelihood that the base has been called correctly. The methods of the present invention are robust and uniformly optimal regardless of the experimental conditions.

According to one aspect of the invention, a computer system is used to identify an unknown base in a sample nucleic acid sequence by the steps of: inputting a plurality of hybridization probe intensities, each of the probe intensities corresponding to a nucleic acid probe; for each of the plurality of probe intensities, determining a probability that the corresponding nucleic acid probe best hybridizes with the sample nucleic acid sequence; and calling the unknown base according to the nucleic acid probe with the highest associated probability.

According to another aspect of the invention, an unknown base in a sample nucleic acid sequence is called by a base call with the highest probability of correctly calling the unknown base. The unknown base in the sample nucleic acid sequence is identified by the steps of: inputting multiple base calls for the unknown base, each of the base calls having an associated probability which represents a confidence that the unknown base is called correctly; selecting a base call that has a highest associated probability; and calling the unknown base according to the selected base call. The multiple base calls are typically produced from multiple experiments. The multiple experiments may be performed on the same chip utilizing different parameters (e.g., nucleic acid probe length).

According to yet another aspect of the invention, an unknown base in a sample nucleic acid sequence is called according to multiple base calls that collectively have the highest probability of correctly calling the unknown base. The unknown base in the sample nucleic acid sequence is identified by the steps of: inputting multiple probabilities for each possible base for the unknown base, each of the probabilities representing a probability that the unknown base is an associated base; producing a product of probabilities for each possible base, each product being associated with a possible base; and calling the unknown base according to a base associated with a highest product. The multiple base calls are typically produced from multiple experiments. The multiple experiments may be performed on the same chip utilizing different parameters (e.g., nucleic acid probe length).

According to another aspect of the invention, both strands of a DNA molecule are analyzed to increase the accuracy of identifying an unknown base in a sample nucleic acid sequence by the steps of: inputting a first base call for the unknown base, the first base call determined from a first nucleic acid probe that is equivalent to a portion of the sample nucleic acid sequence including the unknown base; inputting a second base call for the unknown base, the second base call determined from a second nucleic acid probe that is complementary to a portion of the sample nucleic acid sequence including the unknown base; selecting one of the first or second nucleic acid probes that has a base at an interrogation position which has a high probability of producing correct base calls; and calling the unknown base according to the selected one of the first or second nucleic acid probes.

A further understanding of the nature and advantages of the inventions herein may be realized by reference to the remaining portions of the specification and the attached drawings.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. **1** illustrates an example of a computer system used to execute the software of the present invention;

FIG. **2** shows a system block diagram of a typical computer system used to execute the software of the present invention;

FIG. **3** illustrates an overall system for forming and analyzing arrays of biological materials such as DNA or RNA;

FIG. **4** is an illustration of the software for the overall system;

FIG. **5** illustrates the global layout of a chip formed in the overall system;

FIG. **6** illustrates conceptually the binding of probes on chips;

FIG. **7** illustrates probes arranged in lanes on a chip;

FIG. **8** illustrates a hybridization pattern of a target on a chip with a reference sequence as in FIG. **7**;

FIG. **9** illustrates the high level flow of the probability base calling method; and

FIG. **10** illustrates the flow of the maximum probability method;

FIG. **11** illustrates the flow of the product of probabilities method; and

FIG. **12** illustrates the flow of the wild-type base preference method.

DESCRIPTION OF THE PREFERRED EMBODIMENT

Contents

I. General

In the description that follows, the present invention will be described in reference to a Sun Workstation in a UNIX environment. The present invention, however, is not limited to any particular hardware or operating system environment. Instead, those skilled in the art will find that the systems and methods of the present invention may be advantageously applied to a variety of systems, including IBM personal computers running MS-DOS or Microsoft Windows. Therefore, the following description of specific systems are for purposes of illustration and not limitation.

FIG. **1** illustrates an example of a computer system used to execute the software of the present invention. FIG. **1** shows a computer system **1** which includes a monitor **3**, screen **5**, cabinet **7**, keyboard **9**, and mouse **11**. Mouse **11** may have one or more buttons such as mouse buttons **13**. Cabinet **7** houses a floppy disk drive **14** and a hard drive (not shown) that may be utilized to store and retrieve software programs incorporating the present invention. Although a floppy disk **15** is shown as the removable media, other removable tangible media including CD-ROM and tape may be utilized. Cabinet **7** also houses familiar computer components (not shown) such as a processor, memory, and the like.

FIG. **2** shows a system block diagram of computer system **1** used to execute the software of the present invention. As in FIG. **1**, computer system **1** includes monitor **3** and keyboard **9**. Computer system **1** further includes subsystems such as a central processor **52**, system memory **54**, I/O controller **56**, display adapter **58**, serial port **62**, disk **64**, network interface **66**, and speaker **68**. Other computer systems suitable for use with the present invention may include additional or fewer subsystems. For example, another computer system could include more than one processor **52** (i.e., a multi-processor system) or memory cache.

Arrows such as **70** represent the system bus architecture of computer system **1**. However, these arrows are illustrative of any interconnection scheme serving to link the subsystems. For example, speaker **68** could be connected to the other subsystems through a port or have an internal direct connection to central processor **52**. Computer system **1** shown in FIG. **2** is but an example of a computer system suitable for user with the present invention. Other configurations of subsystems suitable for use with the present invention will be readily apparent to one of ordinary skill in the art.

The VLSIPS technology provides methods of making very large arrays of oligonucleotide probes on very small chips. See U.S. Pat. No. 5,143,854 and PCT patent publication Nos. WO 90/15070 and 92/10092, each of which is incorporated by reference for all purposes. The oligonucleotide probes on the "DNA chip" are used to detect complementary nucleic acid sequences in a sample nucleic acid of interest (the "target" nucleic acid).

The present invention provides methods of analyzing hybridization intensity files for a chip containing hybridized nucleic acid probes. In a representative embodiment, the files represent fluorescence data from a biological array, but the files may also represent other data such as radioactive intensity data. Therefore, the present invention is not limited to analyzing fluorescent measurements of hybridizations but may be readily utilized to analyze other measurements of hybridization.

For purposes of illustration, the present invention is described as being part of a computer system that designs a chip mask, synthesizes the probes on the chip, labels the nucleic acids, and scans the hybridized nucleic acid probes. Such a system is fully described in U.S. patent application Ser. No. 08/249,188, now U.S. Pat. No. 5,591,639 which has been incorporated by reference for all purposes. However, the present invention may be used separately from the overall system for analyzing data generated by such systems.

FIG. **3** illustrates a computerized system for forming and analyzing arrays of biological materials such as RNA or DNA. A computer **100** is used to design arrays of biological polymers such as RNA or DNA. The computer **100** may be, for example, an appropriately programmed Sun Workstation or personal computer or workstation, such as an IBM PC equivalent, including appropriate memory and a CPU as shown in FIGS. **1** and **2**. The computer system **100** obtains inputs from a user regarding characteristics of a gene of interest, and other inputs regarding the desired features of the array. Optionally, the computer system may obtain information regarding a specific genetic sequence of interest from an external or internal database **102** such as GenBank. The output of the computer system **100** is a set of chip design computer files **104** in the form of, for example, a switch matrix, as described in PCT application WO 92/10092, and other associated computer files.

The chip design files are provided to a system **106** that designs the lithographic masks used in the fabrication of